Reproductive, cytological and biochemical toxicity of fenugreek in male Swiss albino mice

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Fenugreek was evaluated for its effects on reproductive, cytological and biochemical toxicity in mice. On the basis of evaluated maximum tolerated dose (MTD; 9.77 g/kg), the present doses selected for sub-chronic (90 days) treatment were 153, 305 and 610 mg/kg/day by oral gavage corresponding to 1/64, 1/32 and 1/16, of MTD, respectively. Total sperm count, motility and spermatozoa morphology was screened. Cytological changes in testicular chromosomes and pregnancy rate in untreated females after mating with treated males were estimated. In testicular cells, total proteins, nucleic acids, malondialdehyde (MDA) and non-protein sulfhydryl (NP-SH) and serum hormonal levels were estimated. The fenugreek treatment particularly with higher dose caused significant changes in the percent motility, sperm count, spermatozoa morphology, chromosomal aberrations, rate of pregnancy and pre-implantation loss. Male fertility was decreased in higher treated doses. In testicular cells, nucleic acids and NP-SH were depleted while MDA levels increased. In conclusion, fenugreek administration at higher dose induced toxicity including teratogenic, foetotoxic, reproductive changes and the abnormal shapes of the sperms. In view of the observed oxidant potentials, present study paves a path to further investigate its clinical effects on reproductive system.

Key words: Fenugreek; reproductive toxicity; spermatozoa; sperm count; oxidative stress.

INTRODUCTION

The dried ripe seeds, leaves and their extracts from Trigonella foenum-graecum L. (fenugreek) have been extensively used as a source of anti-diabetic (Thakran et al., 2004; Kumar et al., 2005; Baquer et al., 2011), hypcholesterolaemic (Abdel-Barry et al., 1997), antifungal (Haouala et al., 2008), anti-bacterial (Sudar and Kirti, 2006), immunomodulatory (Ramesh et al., 2002; Bin-Hafeez et al., 2003), anti-inflammatory and antipyretic (Ahmadiani et al., 2001). The chemical composition of fenugreek includes alkaloids (trigonelline, choline, gentianine and carpane), diosgenin, saponins, steroid sapogenins, trigoneoside Ia, Ib, Ila, Ilb,IIa and IIb, glycoside and trifoenoside A, flavonoids, tannins, including quercetin, vitexin, fixed oils (Petit et al., 1995; Skibola and Smith, 2000). Seeds contain amino acid 4-hydroxyisoleucine (4-OH-Ile), (4, 5-dimethyl-3-hydroxy-2[5H]-furanone), (Flammang et al., 2004).

Although fenugreek has traditionally been considered safe and well tolerated, some toxic effects have been associated with its use such as transient diarrhea, flatulence, mild hepatitis and dizziness (Abdel-Barry and Al-Hakiem, 2000).

It has also been considered as allergenic (Faeste et al., 2009) and anti-fertility in rabbits (Kassem et al., 2006). Quercetin has been shown to cause abnormal testes with necrosis, apoptosis of spermatogonia, delay in the development of seminiferous tubes in Xenopus laevis (Cong et al., 2006). Quinones and trigonelline have been found to be cytotoxic and are mutagenic in bacteria (Wu et al., 1997), act as pro-oxidants (Skibola and Smith,
2000), depletion of serum triiodothyronine (T3) concentration and T3/T4 ratio, but increases thyroxine (T4) levels both in mice and rats (Panda et al., 1999). Fenugreek seeds have been shown to possess estrogenic activity that disturbs the endometrial lining system and interferes with fetal development (Kassem et al., 2006; Sreeja et al., 2010), prominent congenital disorders including hydrocephalus, anencephaly, cleft palate and spina bifida were reported among women who consumed fenugreek seeds during pregnancy (Skalli, 2006). Khalqi et al. (2010) reported that fenugreek supplementation 1 g/kg/day to pregnant females during gestation period, decreased litter size, increase pup mortality, reduced body weights and formation of cleft palate and a bump on head in newborns. Another research group, Mozaffari et al. (2010) highlighted that fenugreek treatment (3.2 g/kg) to pregnant rats caused severe adverse alterations in rat fetus such as disorder in developing hind limb long bone. However, Aswar et al. (2010) have reported that furostanol glycosides fraction of fenugreek did not change testosterone level and anorgenic activity in rats.

Although pharmacological effects of fenugreek have been reported by a large number of research groups, little is known about the influence of fenugreek on the male reproductive, cytological and biochemical effects. The present study was undertaken to evaluate male reproductive, cytological, biochemical changes after sub-chronic (90-days) treatments with different doses of fenugreek to male Swiss albino mice. The rationale of this study is based on the adverse effects of the constituents of fenugreek on male reproduction system and a paucity of literature on toxicity of fenugreek as food supplement.

MATERIALS AND METHODS

Fenugreek

Commercially available fenugreek in the form of capsules was used in the present study (Nature's Way Products, Inc., Springville, Utah 84663, USA). Each capsule contained 1.22 g of fenugreek seed and two capsules recommended for human use.

Dose, route and duration of treatment

The dose of fenugreek was determined by maximum tolerated dose (MTD) and human therapeutic dose with reference to the surface area rule. On basis of evaluated MTD (9.77 g/kg), the doses of fenugreek selected for sub-chronic study were 153, 305 and 610 mg/kg/day corresponding to 1/64, 1/32 and 1/16, of MTD, respectively (Eaton and Klassen, 1996). The human therapeutic dose of fenugreek is 2.44 g per adult human weighing 60 kg. According to the rule of surface area ratio of mice (20 g) and man (60 kg), the calculated ratio is 0.0026 and the dose of fenugreek on mice would be (0.0026 x 100 x 50 = 13.0 mg/kg). The dose used experimentally is generally six times more than the calculated value (13 x 6 = 78 mg/kg/day). This is because the metabolic rate is more in mouse as compared to human being (Eaton and Klassen, 1996). The doses were selected on the basis of human therapeutic dose and the surface area rule. Aqueous suspension of fenugreek was administered by gastric intubation (oral) daily for a period of 90 days. The mice in the control group were given tap water as the vehicle.

Animals

Male and female Swiss albino mice (SWR) aged 6–8 weeks and weighing 25–30 g was obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The animals were fed on Purina chow diet (Manufactured by Grain Silos and Flour Mills Organization, Riyadh, Saudiarabia) and water ad libitum. They were maintained under standard conditions of humidity (50-55%), temperature (23-25°C), and light (12 h light/12 dark cycles). All procedures including euthanasia procedure were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (1996) as well as the Ethical Guidelines of the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Experimental design

The experimental groups of mice consisted of the following four groups: Group 1, control (0.3 mL/mouse, tap water); 2, fenugreek (153 mg/kg/day); 3 fenugreek (305 mg/kg/day) and 4, fenugreek (610 mg/kg/day). Each of the following parameters were evaluated in the above four groups (the total number of mice used is mentioned between the parenthesis against each parameter): Reproductive organ weight, motility and count of sperm (20 mice); spermatozoa morphology (20 mice); the rate of pregnancy and mean implants (40 male mice and 120 female mice in each week of the mating); cytology of the testes chromosomes (20 mice); biochemical study on estimation of proteins, nucleic acids, malondialdehyde (MDA) and non-protein sulfhydril (NP-SH) in testicular cells (20 mice).

Evaluation of spermatozoa motility, count and abnormalities

The spermatozoa were obtained by making small cuts in caudae epididymis and vas deferens placed in 1 ml of modified Krebs Ringer-bicarbonate buffer (pH 7.4). The sperm suspension was evaluated for sperm content and percent motility. The percent motility was determined by the progressive and non-progressive movements of sperm observed under a compound microscope (Laborlux 11, Leitz, Germany) (Anderson et al., 1983). The sperm count was determined under a Neubauer haemocytometer (Superior, Marientfeld, Germany) (Al-Shabanah, 1997). To evaluate the spermatozoa abnormalities, the sperm suspension was stained with eosin; smears were made on slides, air-dried and made permanent. Coded slides were examined by bright field microscope with an oil immersion lens. The different spermatozoa abnormalities screened were amorphous, banana shaped, swollen chromosome, triangular head, macrocephali and rotated head screened (Al-Majed et al., 2006).

Cytological analysis of germ cells

The protocol described in a study of chromosomal aberrations in the testis (Al-Shabanah, 1997) was followed. The mice were killed after the last day of the treatment, testes were removed in an isotonic sodium citrate solution and the seminiferous tubules were teased to form a cell suspension. The suspension was centrifuged and the pellet re-suspended in the hypotonic citrate solution. After the second centrifugation the supernatant was discarded and the
pellet suspended in a fixative (methanol and acetic acid, 3:1). The chromosomal preparations were made by the air drying technique. The coded slides were stained in Giemsa solution and screened for the aberrations including aneuploids, autosomal univalents, sex-univalents and polyploids.

Studies on rate of pregnancy and mean implants per female mouse

The methods described in male anti-fertility study and dominant lethal assay (Al-majed et al., 2006) were followed to evaluate the: (i) Rate of fertility in male mice, (ii) induction of pregnancy, (iii) total and pre-implantation loss and (iv) embryo-toxicity. After the treatment, each male mouse in the treated and control groups was caged with three female mice, which were allowed to stay with the male for 1 week. The female mice were examined 13 days following their caging and presumptive mating and the number of pregnant mice was recorded to determine percent fertility. The pre-implantation loss was calculated by comparing the number of implantations per pregnant female in the treated and control groups. The dead implants per pregnant female were determined to obtain the post-implantation embryonic loss (Green et al., 1985).

Estimation of total proteins and nucleic acids

Total proteins in testes were estimated by using a modified Lowry method (Schacterle and Pollack, 1973). Bovine serum (Merck, Darmstadt, Germany) albumin was used as standard. The method described by Bregman (1983) was used to determine the levels of nucleic acids (DNA and RNA). To determine the levels of nucleic acids, testes were homogenized and the homogenate was suspended in ice-cold trichloroacetic acid (TCA). After centrifugation (500 × g for 3 min at 23°C), the pellet was extracted with ethanol. The levels of DNA were determined by treating the nucleic acid extract with diphenylamine reagent and reading the intensity of blue color at 600 nm. For quantification of RNA, the nucleic acid extract was treated with orcinol and the green color was read at 660 nm. Standard curves were used to determine the amounts of nucleic acids present.

Determination of MDA concentrations

The method described by Ohkawa et al. (1979) was used to estimate lipid peroxidation product MDA in testes. Testes were homogenized in TCA solution and the homogenate suspended in thiobarbituric acid. After centrifugation the optical density of the clear pink supernatant was read at 532 nm. Malondialdehyde bis (dimethyl acetal) was used as an external standard.

Quantification of the NP-SH levels

The method described by Sedlak and Lindsay, (1968) was used to determine the levels of NP-SH. The testes were homogenized in ice-cold 0.02 M ethylene-α-amine tetra acetic acid disodium (EDTA) and mixed with TCA. The homogenate was centrifuged at 3000 × g. The supernatant was suspended in tris buffer, 5·5′-dithiobis-(2 nitrobenzoic acid) (DTNB) and read at 412 nm against reagent blank with no homogenate.

Estimation of hormones in the serum

The plasma samples were analyzed to determine the concentration of different hormones (human chorionic gonadotropin, progesterone, leutinizing hormone, follicle stimulating hormone, estradiol, prolactin and testosterone). The analysis was carried by direct immunoenzymatic calorimetric method based on ELISA. The protocol used for each hormone was according to the methods described for the particular kit (DIA, METRA, Italy).

Statistical analysis

The means of the four groups were compared using analysis of variance (ANOVA). Tukey-Kramer’s post hoc comparison test was used to locate specific group differences. The percentage of fertility and dead embryos of four groups were compared using the χ²-test. The two-tailed 0.05 level of significance was used for all data analysis. Data were analyzed using SPSS version10.0 (SPSS Inc., Chicago, IL, USA) software.

RESULTS

Spermatozoa motility, count and abnormalities caused after sub-chronic (90 days) treatments with different doses (153, 305 and 610 mg/kg/day) of fenugreek to male SWR mice shown in Table 1. Higher two doses caused a significant (P<0.05) reduction in the sperm count compared to control, respectively. Percent sperm motility was also significantly reduced in mice treated with fenugreek by medium (P<0.01) and high (P<0.001) doses for 90 days as compared to the values obtained in the control group. The sub-chronic treatment with high dose (610 mg/kg/day) fenugreek significantly increased the banana shaped (P<0.05), triangular head (P<0.01) and the total abnormalities (P<0.05) as compared to control group. The sub-chronic treatment with higher dose of fenugreek induced a significant (P<0.05) increase in frequency of aneuploids, autosomal univalents and sex-univalents compared to control group. The frequency of polyploids and total-percent aberrations was significantly increased at higher doses (305 and 610 mg/kg/day) after prolonged treatment of fenugreek (Table 2).

In mating week 1, the sub-chronic treatment of male mice with fenugreek significantly (P<0.05) decreased the percent pregnant female mice at a dose of 610 mg/kg/day. At the same dose, there was a significant reduction in the mean total implants per pregnant female mice (P<0.05) and mean live implants per pregnant female mice (P<0.01). After sub-chronic treatment with higher dose of fenugreek, a significant increase was found in the mean dead implants per pregnant female mice (P<0.05) and the percent dead embryos (P<0.001) as compared to the values obtained in the control mice (Table 3). In mating week 2, the sub-chronic treatment of male mice with fenugreek (610 mg/kg/day) caused a significant reduction in the mean total implants per pregnant female mice (P<0.05) and mean live implants per pregnant female mice (P<0.01). A significant increase was found in the mean dead implants per pregnant female mice (P<0.05) and the percent dead embryos (P<0.01). There was a visible reduction (96.43 to 70.00%) in pregnant female mice in the mating week 2,
Table 1. Effect of fenugreek on sperm motility, count and abnormality in Swiss albino mice after sub-chronic treatment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (tap water, 0.3 ml/mouse/day)</th>
<th>Fenugreek (153 mg/kg/day)</th>
<th>Fenugreek (305 mg/kg/day)</th>
<th>Fenugreek (610 mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent sperm motility and sperm count (log N/mm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent sperm motility</td>
<td>98.00±1.23</td>
<td>92.00±2.55</td>
<td>85.00±3.16**</td>
<td>83.00±2.55***</td>
</tr>
<tr>
<td>Sperm count</td>
<td>4.98±0.07</td>
<td>4.88±0.03</td>
<td>4.77±0.06*</td>
<td>4.67±0.08*</td>
</tr>
<tr>
<td>Percent sperm abnormalities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amorphous</td>
<td>0.49±0.07</td>
<td>0.34±0.06</td>
<td>0.49±0.07</td>
<td>0.86±0.20</td>
</tr>
<tr>
<td>Banana shaped</td>
<td>0.44±0.06</td>
<td>0.30±0.05</td>
<td>0.55±0.11</td>
<td>1.06±0.24*</td>
</tr>
<tr>
<td>Swollen achromosome</td>
<td>0.30±0.08</td>
<td>0.41±0.06</td>
<td>0.39±0.12</td>
<td>0.71±0.24</td>
</tr>
<tr>
<td>Triangular head</td>
<td>0.29±0.07</td>
<td>0.45±0.07</td>
<td>0.38±0.06</td>
<td>0.65±0.09**</td>
</tr>
<tr>
<td>Macrocephali</td>
<td>0.19±0.05</td>
<td>0.28±0.03</td>
<td>0.44±0.06*</td>
<td>0.32±0.08</td>
</tr>
<tr>
<td>Rotated head</td>
<td>0.10±0.04</td>
<td>0.15±0.04</td>
<td>0.16±0.05</td>
<td>0.19±0.06</td>
</tr>
<tr>
<td>Total abnormalities</td>
<td>1.82±0.39</td>
<td>1.94±0.12</td>
<td>2.43±0.29</td>
<td>3.79±0.76*</td>
</tr>
<tr>
<td>Total sperms screened</td>
<td>5050</td>
<td>5100</td>
<td>4950</td>
<td>5000</td>
</tr>
</tbody>
</table>

Values are means ± S.E. (n = 6). Significant differences are indicated by *p < 0.05, **P<0.01 and ***P<0.001 when compared with control animals (One-way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done individually for different parameters).

Table 2. Effect of Fenugreek on testis chromosomes in Swiss albino mice after sub-chronic treatment.

<table>
<thead>
<tr>
<th>Different abnormalities screened/Total</th>
<th>Control (tap water, 0.3 ml/mouse/day)</th>
<th>Fenugreek (153 mg/kg/day)</th>
<th>Fenugreek (305 mg/kg/day)</th>
<th>Fenugreek (610 mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aneuploids</td>
<td>3.99 ± 0.49</td>
<td>4.79 ± 0.76</td>
<td>4.70 ± 0.62</td>
<td>6.28 ± 0.69*</td>
</tr>
<tr>
<td>Autosomal univalents</td>
<td>3.22 ± 0.63</td>
<td>4.09 ± 0.70</td>
<td>4.66 ± 0.71</td>
<td>6.0 ± 0.94*</td>
</tr>
<tr>
<td>Sex-univalents</td>
<td>2.80 ± 0.48</td>
<td>4.03 ± 1.02</td>
<td>3.82 ± 0.78</td>
<td>5.49 ± 0.95*</td>
</tr>
<tr>
<td>Polyploids</td>
<td>2.94 ± 0.39</td>
<td>4.18 ± 0.48</td>
<td>5.57 ± 0.76*</td>
<td>5.89 ± 0.92*</td>
</tr>
<tr>
<td>Total-percent aberrations</td>
<td>12.93 ± 1.23</td>
<td>17.09±2.22</td>
<td>18.77 ± 1.59*</td>
<td>23.68 ± 2.36**</td>
</tr>
<tr>
<td>Total stages screened</td>
<td>550</td>
<td>530</td>
<td>500</td>
<td>520</td>
</tr>
</tbody>
</table>

Values are means ± S.E. (n = 6). Significant differences are indicated by *p < 0.05, **P<0.01 and ***P<0.001 when compared with control animals (One-way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done individually for different parameters).

but the decrease was statistically non-significant (P>0.05) as compared to the values obtained in the control mice (Table 3). The plasma estradiol levels increased while the testosterone levels decreased in fenugreek (610 mg/kg/day) treated mice and the values were statistically compared with control group (Table 4). Sub-chronic treatment with high dose (610 mg/kg/day) of fenugreek resulted in significant (P<0.05) increase in testicular MDA and decrease in NP-SH levels as compared to the values obtained in the control group (Figure 1). The 90 days treatment with high dose of fenugreek was found to inhibit the testicular levels of RNA (P<0.01) and DNA (P<0.05) significantly when compared to control group of mice (Figure 2).

DISCUSSION

The sub-chronic oral administration of fenugreek at the higher doses of 305 and 610 mg/kg body weight per day has caused male reproductive toxicity followed by reduced fertility, decreased motility, sperms count and an increase in the proportion of abnormal sperms in mice. The reduction observed in the fertility of male mice and the abnormal shapes of the sperms observed might be related with the increased accumulation of free radicals. Previous studies (Farag et al., 2010; Al-Majed et al., 2006) also showed that the depletion of glutathione cause spermatotoxicity and produce abnormal shapes of the sperms. Furthermore, the endocrinological study in plasma of male mice showed increase of estradiole and reduction of testosterone, which are known to affect the fertility. These results confirms the previous reports (Kassem et al., 2006; Khare et al., 1983) which showed fenugreek seeds to demonstrate an anti-fertility effect in both male and female rabbits. There was a significant reduction of total and live implants per pregnant female mice and an increase of dead implants per pregnant female mice and the percent dead embryos. These results suggest an embryo-foetal toxicity effect of the
fenugreek. This is similar to the previous findings, such as Savin essential oil extract and Acanthus montanus aqueous extract affect fertility either by promoting anti-implantation (Chamorro et al., 1990) or through embryonic loss or re-absorption (Asonglem et al., 2008). This is in agreement with earlier work that showed decrease of fetal size and an increase in fetal mortality rate after receiving a single intra peritoneal injection of a decoction from fenugreek leaves on day 10 in rats (Araee et al., 2009). Khalki et al. (2010) reported that aqueous seeds extract of Trigonella foenum-graecum affected reproduction in mice and showed teratogenic and foetotoxic effect. Steroidal saponins and alkaloids have been shown to be teratogenic. Recently, Araee et al. (2009) has considered that in view of the presence of the steroidal saponin diosgenin in fenugreek seeds, it is likely that the administration of fenugreek in high doses adversely influences bone marrow cell proliferation. Similarly, Incardona et al. (1998) demonstrated a teratogenic effect of alkaloids from Veratrum californicum in chick embryos. Accordingly, we suggest that these compounds could be responsible for the observed teratogenic effects in mice in our investigation.

Our study on biochemical analysis showed an increase in the levels of MDA and depletion of NP-SH, RNA and DNA. These results demonstrate that the oxidative stress in testicular milieu is associated with DNA damage and produces higher frequency of abnormal sperms with significant effect on male fertility. These changes are attributed to the toxic constituents present in fenugreek, such as quinones and flavonoids (Skibola and Smith, 2000; Wu et al., 1997). Previous report (Kumar et al., 2002) found the links between free radical species and DNA damage, sperm abnormality and reduced fertility in male mice. The present results confirm an earlier report (Skibola and Smith, 2000) which showed that these compounds cross the placenta and place the unborn fetus at great risk. It has shown that exposure of X. laevis to quercetin was found to cause abnormal testes, including necrosis, apoptosis of spermatogonia, delay in the development of seminiferous tubules (Cong et al., 2006). The treatment with fenugreek was also found to induce significant changes in the chromosomal aberrations after sub-chronic treatment. This induction of chromosomal aberrations might be related to the oxidant constituents of fenugreek. The mode of action of fenugreek on the observed reproductive, cytological and biochemical changes is not known. Nevertheless, it may be related with the generation of oxygen radicals by some of the toxic constituents of fenugreek (Wu et al., 1997; Kumar and Muralidhara 1999; Skibola and Smith, 2000; Cong et al., 2006).

Nevertheless, there are some important observations on the antioxidant and/or protection activity of pre-treatment with repeated doses of fenugreek in experimental animals. These studies revealed protection against diabetes (Thakran et al., 2004; Kumar et al., 2005; Baquer et al., 2011), hypercholesterolemia (Abdel-Barry et al., 1997), fungal or bacterial infections (Haouala et al., 2008; Sudar and Kirti, 2006) and also showed the potential effects as immunomodulatory (Ramesh et al., 2002; Bin-Hafeez et al., 2003), anti-inflammatory and antipyretic (Ahmadiani et al., 2001). Previous reports have suggested that cell sensitivity to the source of exposure may reflect differences in the metabolism and the intracellular concentrations of enzymes and mediators of various target biochemical processes or repair mechanisms (Al-Harbi et al., 1995; Lahdetie et al., 1997). Furthermore, the protocol used in most of these studies is the continuous pre-loading of fenugreek before the exposure of the tissues to the oxidant injury. The difference in duration and frequency of exposures might have determined the proportion of antioxidant/oxidant ratio and the related outcome. Taken together, the observed changes of fenugreek might be related to its effect on the increase of MDA and depletion of NP-SH. The exact mechanism of action is not known.

### Table 3. Effect of Fenugreek on the induction of dominant lethal mutations after sub-chronic treatment in male Swiss albinino mice.

<table>
<thead>
<tr>
<th>Treatment and dose (mg/kg. Body weight/day)</th>
<th>Mating week 1</th>
<th>Mating week 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pregnant females</td>
<td>Mean Implants/pregnant female ± S.E.</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>Live</td>
</tr>
<tr>
<td>Control (0.3 ml tap water/mouse/day)</td>
<td>29/30 (96.67)</td>
<td>11.00±0.36</td>
</tr>
<tr>
<td>Fenugreek (153 mg/kg/day)</td>
<td>26/30 (86.67)</td>
<td>10.77±0.60</td>
</tr>
<tr>
<td>Fenugreek (305 mg/kg/day)</td>
<td>27/28 (96.43)</td>
<td>9.63±0.64</td>
</tr>
<tr>
<td>Fenugreek (610 mg/kg/day)</td>
<td>20/28 (71.43)*</td>
<td>8.90±0.57*</td>
</tr>
</tbody>
</table>

Values are means ± S.E. (n = 6). Significant differences are indicated by *P < 0.05, **P<0.01 and ***P<0.001 when compared with control animals (One-way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done individually for different parameters).
Table 4. Effect of fenugreek on certain pituitary-gonadal hormones in plasma of male and female Swiss albino mice after sub-chronic treatment.

<table>
<thead>
<tr>
<th>Determination of pituitary-gonadal hormones in plasma</th>
<th>Control (tap water, 0.3 ml/mouse/day)</th>
<th>Fenugreek (153 mg/kg/day)</th>
<th>Fenugreek (305 mg/kg/day)</th>
<th>Fenugreek (610 mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human-chorionic gonadotropin</td>
<td>0.97±0.05</td>
<td>1.00±0.09</td>
<td>0.96±0.04</td>
<td>0.98±0.55</td>
</tr>
<tr>
<td>Leutenizing hormone</td>
<td>1.70±0.12</td>
<td>1.60±0.07</td>
<td>1.80±0.15</td>
<td>1.74±0.05</td>
</tr>
<tr>
<td>Follicle-Stimulating Hormone</td>
<td>1.50±0.08</td>
<td>1.50±0.03</td>
<td>1.65±0.08</td>
<td>1.43±0.07</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.28±0.013</td>
<td>0.29±0.04</td>
<td>0.29±0.01</td>
<td>0.40±0.03*</td>
</tr>
<tr>
<td>Prolactin</td>
<td>0.46±0.02</td>
<td>0.47±0.04</td>
<td>0.47±0.02</td>
<td>0.55±0.03</td>
</tr>
<tr>
<td>Testosterone</td>
<td>18.70±1.13</td>
<td>18.00±1.00</td>
<td>17.20±1.12</td>
<td>15.00±0.76*</td>
</tr>
</tbody>
</table>

Values are means ± S.E. (n = 6). Significant differences are indicated by *p < 0.05, **P<0.01 and ***P<0.001 when compared with control animals (One-way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done individually for different parameters).

Figure 1. Effect of Fenugreek on MDA and NP-SH levels in testes of mice after sub-chronic treatment. Values are means ± S.E. (n = 6). Significant differences are indicated by *p < 0.05, **P<0.01 and ***P<0.001 when compared with control animals (One-way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done individually for different parameters).
Figure 2. Effect of fenugreek DNA, RNA and total protein levels in testes of mice after sub-chronic treatment. Values are means ± S.E. (n = 6). Significant differences are indicated by *p < 0.05, **P<0.01 and ***P<0.001 when compared with control animals (One-way ANOVA and Post hoc Tukey-Kramer multiple comparison test was done individually for different parameters).
however, it appears to be related with the influence of fenugreek on CYPs, resulting in the accumulation of free radical species. Given that increasing number of people are exposed to herbal preparations that contain many constituents with potential of CYP modulation, our observation on the related oxidative potentials warrants careful use of fenugreek.

**ABBREVIATIONS**

4-OH-Ile, Amino acid 4-hydroxysolucele; MTD, maximum tolerated dose; TCA, trichloroacetic acid; EDTA, ethylene-o-amine tetra acetic acid disodium; DTNB, 5,5′-dithiobis-(2 nitrobenzoic acid).

**REFERENCES**


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